Title: Introduction to the Symposium – Keeping Time During Evolution: Conservation and Innovation of the Circadian Clock

Authors: Ann M. Tarrant[*,#] and Adam M. Reitzel[+]

[#] Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543

[+] Department of Biology, University of North Carolina at Charlotte, Charlotte, NC 28223

* Corresponding author:

Email: atarrant@whoi.edu

Phone: 508-289-3398

Fax: 508-457-2134

SYNOPSIS

Diurnal and seasonal cues play critical and conserved roles in behavior, physiology, and reproduction in diverse animals. The circadian clock is a transcription-translation feedback loop that represents the molecular mechanism underlying many of these periodic processes, frequently through responses to light. Although much of the core regulatory machinery is deeply conserved among diverse animal lineages, there are also many examples of innovation in the way the clock either is constructed at the molecular-level or deployed in coordinating behavior and physiology. The nine papers contained within this issue address aspects of circadian signaling in diverse taxa, utilize wide-ranging approaches, and collectively provide thought-provoking discussion of future directions in circadian research.

Introduction

Diurnal and seasonal cues play critical and conserved roles in behavior, physiology, and reproduction in diverse animals. The circadian clock is a transcription-translation feedback loop that represents the molecular mechanism underlying many of these periodic processes, frequently through responses to light. Current understanding of the molecular mechanisms that constitute the animalian circadian clock has stemmed largely from two groups of animals: mammals and dipteran insects (particularly *Drosophila melanogaster*). Broadly, because many core components of the clock and their protein-level interactions are shared between these two lineages, it may be inferred that these regulatory pathways date at least back to the common ancestor of the protostomes and deuterostomes.

Studies conducted on mammals and *Drosophila* have provided a tremendous depth of data about the core proteins of the clock, their interactions in regulating 24-hour rhythms, and their relative conservation in bilaterian evolution. However, an emerging theme from recent studies of circadian processes is that the genes involved in rhythmic responses can vary considerably from the components of the clock as defined in these traditional model organisms. Our understanding of the degree of conservation of circadian mechanisms in non-model animals and of the innovation both of the molecular regulation and the function(s) of the clock has traditionally been patchy, and often very limited**.** A recent explosion of circadian research in diverse animals, particularly non-dipteran insects and marine species, has begun to fill these gaps. New advances in sequencing technology promise to provide exceptional opportunities for deeper insights into the molecular composition of the clock and into the gene networks regulated

by circadian clocks, particularly those related to physiology, behavior, and reproduction. Additionally, recent studies have revealed environmental inputs besides light that can entrain the circadian clock and have provided an increasing understanding of myriad functions of the circadian clock. Hence, we convened a symposium to highlight research that exemplifies advances in these two complementary areas of circadian biology (conservation of molecular mechanisms throughout the animal kingdom and innovation of the clock for non-canonical roles). The nine papers contained within this issue address aspects of circadian signaling in diverse taxa, utilize wide-ranging approaches, and collectively provide thought-provoking discussion of future directions in circadian research.

I. Conservation of the circadian clock in the evolution of animals

After more than a decade of work on mammals and insects in the 1990s and early 2000s, the conservation of the molecular mechanisms composing the circadian clock in bilaterian animals increasingly became realized. Since then, work on a growing diversity of animals has confirmed the conservation of many of the clock's regulatory mechanisms in other lineages and has also begun to show the divergences stemming from duplication or loss of genes and the influence of phylogenetic position. Studies of the evolution of components of the circadian clock in a phylogenetic framework, coupled with analysis of their functions and interactions, are beginning to provide the necessary data for understanding the evolution of this central gene network in behavior and physiology.

Circadian clocks are present in all major branches of life, prokaryotic and eukaryotic; however, the molecular components differ completely between these distantly related groups,

suggesting that independent evolutionary events have resulted in the circadian clocks observed in extant prokaryotes and eukaryotes. While the clocks of eukaryotes (e.g., plants, fungi, and animals) are largely understood in terms of transcription-translation feedback loops, bacterial clocks are driven by protein-protein interactions over 24-hour cycles. Chang et al. (this volume) describe the technical application of solution-state nuclear magnetic resonance spectroscopy (NMR) for the purpose of understanding the interactions of the three constituent proteins of the oscillator of cyanobacteria. As they describe, the precise understanding of the structure and dynamics of proteins afforded by analyses on NMR provides insights beyond that available using other methods.

Müller et al. (this volume) describe a unique phototransduction system as well as conserved elements of a circadian regulatory system within siliceous sponges. Siliceous sponges are modern members of a metazoan lineage that diverged early and that notably lacks a nervous system. Müller's group has shown that a cryptochrome, a member of a group of light-sensitive proteins typically composing part of the feedback loop in bilaterian clocks, exhibits oscillating transcription over a light:dark cycle. Additional studies conducted by this group have demonstrated that the enzyme nocturnin is upregulated during darkness and may regulate diel cycles in energetic metabolism. While many key elements of the bilaterian circadian clock (e.g., homologs of *Clock* and *Cycle*) have not been identified in sponges, characterization of cryptochromes and nocturnin provide unique insight into the origins of the animal clock.

While studies of circadian regulation in cnidarians are still at an early stage, it is now clear that several elements of the bilaterian circadian clock are present in cnidarians, particularly anthozoans (sea anemones and corals) (Levy et al. 2007; Reitzel et al. 2010; Hoadley et al. 2011). Anthozoans contain homologs of *Clock* and *Cycle*, as well as several cryptochromes.

Light-entrained daily oscillations have been demonstrated for *Clock* and some cryptochromes in anthozoans, suggesting potential conserved functions for these genes in the cnidarian clock. Reitzel et al. (this volume) provide a phylogenetic and experimental analysis identifying the presence of a feedforward loop in the cnidarian *Nematostella vectensis*. For reference, the feedforward loop of *Drosophila* is composed of two PAR-bZIP transcription factors (*vrille* and *pdp1*) that regulate transcription of *Clock* via competitive binding in the promoter region. Reitzel and colleagues identified PAR-bZIP genes in the sea anemone, showed that expression oscillates in a light-dependent manner, and provided *in silico* analysis showing that the *Clock* promoter of *N. vectensis* contains binding sites likely bound by PAR-bZIP transcription factors. Together with previously published data, current research on anthozoan cnidarians suggests not only that many of the core genes composing the bilaterian circadian clock are conserved, but also that some of the protein-DNA interactions may be shared between cnidarians and bilaterians, suggesting an ancient origin for the molecular mechanisms of the circadian clock.

Studies of circadian signaling in arthropods initially focused on *Drosophila*, and given the availability of powerful genetic tools, *Drosophila* continues to be an important circadian model. However, research on other insects (Reppert 2007; Yuan et al. 2007) has revealed that the typical circadian clock in insects may be more similar to mammalian clocks, albeit with some diversification, and the *Drosophila* clock is atypical because of loss of genes. One crucial difference is the presence of only one type of cryptochrome in *Drosophila* (Type I), while most insects have both Type I and Type II. Type II cryptochromes, sometimes referred to as mammalian-type cryptochromes, are direct repressors of the CLOCK:CYCLE dimer. Meuti and Denlinger (this volume) discuss this and other variations in the circadian clocks of insects. They also discuss the potential function of a Type II cryptochrome and other components of the clock

in the daily circadian and seasonal photoperiodic responses of insects, specifically with regard to diapause.

While insects have served as primary models for deciphering the circadian clock in the protostome lineage, additional species from other protostome phyla and subphyla within the arthropods will result in a better characterization of the evolution of the protostome clock. The arthropod Subphylum Chelicerata, which contains spiders, ticks, and horseshoe crabs, represents an important group of organisms that can provide a deeper evolutionary comparison of the composition and function of the circadian clock in arthropods. Battelle (this volume) reports on the role of the circadian clock in the visual system of the horseshoe crab, *Limulus polyphemus*. Her research shows that circadian rhythms, potentially driven by the circadian clock, affect the visual sensitivity of the lateral eyes by increasing their sensitivity at night and decreasing their sensitivity at dawn. This research shows the function of the clock as a modulator of the visual system and not strictly as a time-keeping mechanism.

II. Innovation of the clock for non-canonical roles

Although much of the core regulatory machinery is deeply conserved among diverse animal lineages, there are also many examples of innovation in the way the clock is constructed or used. Genes that are associated with circadian function in some animals may serve a different purpose in other lineages, or may serve multiple roles. For example, Müller et al. (this volume) describe a unique phototransduction system within siliceous sponges in which cryptochromes detect environmental levels of light as well as and bioluminescence. Müller's group has shown

that light may be transmitted from the environment along siliceous spicules throughout the animal.

Circadian clocks may be entrained by a variety of environmental signals or *zeitgebers*, with light being the dominant signal in many habitats. Other cues, such as temperature, availability of food, and interactions between organisms can also entrain clocks, and entrainment varies among taxa. The Merritt laboratory has studied entrainment of the circadian clock in a group of closely related species of flies, commonly called glowworms, which use bioluminescence to attract prey (Maynard and Merritt, this volume). They found that the patterns of entrainment by light varied among species according to habitat. A species that inhabits rainforests exhibits increased activity and bioluminescence at night. In contrast, under conditions of constant darkness, species that inhabit both rainforests and caves exhibit peak luminescence during the afternoon. When these glowworms are found outside of caves, the circadian response is masked by exposure to light. In this case, light serves both to entrain the rhythm and to mask the luminescence. Within this volume, Maynard and Merritt demonstrate for the first time that cave-adapted glowworms are able to use bioluminescence from neighboring conspecifics as a *zeitgeber*. This mechanism allows them to synchronize their foraging, which likely serves both to maximize success in capturing prey and to minimize the risk of cannibalism.

Smarr et al. (this volume) also discusses the importance of masking and social interactions in modulating the response to *zeitgebers*. They describe several examples in which the circadian patterns observed in laboratory studies may differ from patterns in the natural environment. Nocturnal light, which varies on a lunar cycle, can provide a masking cue, such that the time of maximal activity of some nocturnal or crespuscular animals varies throughout the month. Because the presence of competitors or predators can also affect temporal patterns of

activity, the temporal niche of some species is plastic. Rhythmic behavioral activity can become decoupled from other circadian outputs, such as cycles in hormone levels. Thus, to understand how circadian signals respond to environmental entrainment, it is important to consider multiple endpoints.

In many animals, circadian signaling becomes established during early development, even without obvious synchronizing cues. Work in the Spencer laboratory has shown that the heart rate of freshwater turtles varies over a 24-hour cycle (Loudon et al., this volume). The timing of maximum heart rate does not occur at a consistent time of day; rather, it varies among individuals. Within the complex environment of a turtle nest, temperature gradients may provide a synchronizing cue, and animals may detect the vibrations produced by their siblings' heartbeats. Thus, metabolic rhythms within the eggs may enable the embryos to synchronize their development and time of emergence. Loudon et al. (this issue) point out that not all species establish circadian rhythms during early development, and the ability to maintain free-running rhythms varies even among oviparous vertebrates. For example, embryos of several species of snakes maintain metabolic circadian rhythms under constant conditions (Dmi'el 1969) but chicken embryos do not (Akiyama et al. 1999). These examples only hint at the diverse roles that circadian regulation may play within different developmental programs and life histories.

Summary

The talks presented at the symposium and the accompanying papers in this volume highlight exciting new directions in comparative circadian biology. As additional research is conducted on the diversity of clock mechanisms in animals, researchers will be able to generate more specific hypotheses about the evolution of the molecular regulation of the clock. We will

also gain a greater understanding of the suite of functions influenced by the circadian clock and how circadian signaling has been adaptively modified by distinct animal lineages in response to the unique pressures imposed by their habitats and life histories.

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References

Akiyama R, Matsuhisa A, Pearson JT and Tazawa H. 1999. Long-term measurement of heart rate in chicken eggs. Comp Biochem Physiol A 124:483-90.

Battelle B-A. 2013. What the clock tells the eye: Lessons from an ancient arthropod. Integrative and Comparative Biology (Current Issue):xxx-xxx.

Chang YG, Tseng R, Kuo N-W and LiWang A. 2013. NMR of the circadian clock of cyanobacteria. Integrative and Comparative Biology (Current Issue):xxx-xxx.

Dmi'el R. 1969. Circadian rhythm of oxygen consumption of snake embryos. Life Sci 8:1333-41.

Hoadley KD, Szmant AM and Pyott SJ. 2011. Circadian clock gene expression in the coral *Favia fragum* over diel and lunar reproductive cycles. PLoS ONE 6:e19755.

Levy O, Appelbaum L, Leggat W, Gothlif Y, Hayward DC, Miller DJ and Hoegh-Guldberg O. 2007. Light-responsive cryptochromes from a simple multicellular animal, the coral *Acropora millepora*. Science 318:467-70.

Loudon FK, Spencer R-J, Strassmeyer A and Rythm KH. 2013. Metabolic circadian rhythms in embryonic turtles. Integrative and Comparative Biology (Current Issue):xxx-xxx.

Maynard A and Merritt DJ. 2013. Synchronization of circadian bioluminescence as a group-foraging strategy in cave glowworms. Integrative and Comparative Biology (Current Issue):xxx-xxx.

Meuti ME and Denlinger DL. 2013. Evolutionary links between insect circadian and photoperiodic clocks. Integrative and Comparative Biology (Current Issue):xxx-xxx.

Müller WE, Schröder HC, Pisignano D, Markl JS and Wang X. 2013. Metazoan circadian rhythm: Toward an understanding of a light-based zeitgeber in sponges. Integrative and Comparative Biology (Current Issue):xxx-xxx.

Reitzel AM, Behrendt L and Tarrant AM. 2010. Light entrained rhythmic gene expression in the sea anemone *Nematostella vectensis*: the evolution of the animal circadian clock. PLoS ONE 5:e12805.

Reitzel AM, Tarrant AM and Levy O. 2013. Circadian clocks in the Cnidaria: Environmental entrainment, molecular regulation, and organismal outputs. Integrative and Comparative Biology (Current Issue):xxx-xxx.

Reppert SM. 2007. The ancestral circadian clock of monarch butterflies: role in time-compensated sun compass orientation. Cold Spring Harb Symp Quant Biol 72:113-8.

Smarr BL, Schwartz MD, Wotus C and de la Iglesia HO. 2013. Who you callin' diurnal? Redefining temporal niche. Integrative and Comparative Biology (this issue):xx-xx.

Yuan Q, Metterville D, Briscoe AD and Reppert SM. 2007. Insect cryptochromes: gene duplication and loss define diverse ways to construct insect circadian clocks. Mol Biol Evol 24:948-55.